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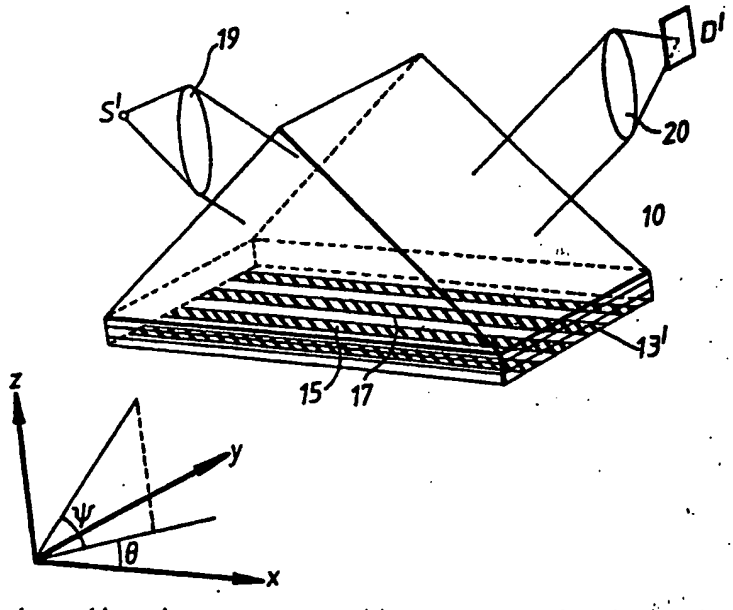

(57) Abstract

This invention discloses a biosensing apparatus comprising: a resonant optical biosensor in which a coating layer thereof is in part sensitized to at least one given assay species and is patterned for causing light diffracton; an extended light source; collimating means arranged to direct light from the extended light source onto the resonant coupled optical biosensor; a light detector; light focussing means arranged to direct light diffracted from the patterned sensitized coating onto the light detector thereby to define a diffraction field in the plane of the detector; and, scanning means arranged to scan the light detector relative to this diffraction field. This invention also discloses a method for detecting optical phase changes during biosensor operation.

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A METHOD FOR DETECTING OPTICAL PHASE CHANGES DURING BIOSENSOR OPERATION, BIOSENSING APPARATUS AND A BIOSENSOR ADAPTED FOR USE IN THE SAME.

TECHNICAL FIELD

The present invention concerns improvements in or relating to the construction and utilisation of optical biosensors, in particular resonant optical biosensors. Such biosensors include a sensitized coating layer which is located in the evanescent region of a resonant field. Interaction between the sensitized coating layer and the assay species to which it is sensitized results in a change in the thickness of the coating layer. This change is made manifest as a change in the optical phase of a resonant signal.

Examples of this type of biosensor are described in published United Kingdom Patent Application Nos. 2173895 and 2174802 as also in co-pending United Kingdom Patent Application No. 8900556.5 (Agents Ref: F20614).

In the first of these references, a metallic layer or metallised grating is interposed between the sensitized coating layer and a light coupling body. The sensitized coating lies in the evanescent region of an optical frequency electron resonance field.

In the second of these references the sensitized coating layer is provided as an integral part of a monitored resonant cavity. Light is coupled into and out of the resonant cavity by means of prism coupling and either a thin low refractive index layer or a multi-layer dielectric mirror formed as an integral part of the mirror resonant cavity. In the last of these references the construction is similar except in that prism coupling is replaced by a grating.

BACKGROUND ART

A resonant optical biosensor 1, similar to that described in the second of the references, UK Patent Application No. 2174802, is depicted in Figure 1 of the drawings. This biosensor 1 includes a mirrored resonant cavity 3 which is abutted to an open ended fluid sample container 5. Light from a light source S is coupled into the resonant cavity 3 by means of a prism 10 of relatively high refractive index n_0. This prism 10 also serves to couple light from the resonant cavity 3 to a light detector D. One mirror of the resonant cavity 3 is provided by a relatively low refractive index n_1 layer 11, of thickness t_1. The body 12 of the cavity is of relatively high refractive index n_2 and of thickness t_2. Light coupling is by frustrated total internal reflection (the mirror layer 11 is thin). The other mirror of the resonant cavity 3 is provided by a relatively low refractive index n_3 layer 13, of thickness t_3. This layer 13 is uniformly thick and is sensitized in its entirety to a given assay species. As shown, this sensitized coating layer 13 is exposed to a fluid sample 14, of refractive index n_4 and thickness t_4. The effect of assay species present in the fluid sample is to provide a incremental change Δt_3 in the thickness t_3 of the sensitized coating layer 13 as the species is absorbed on this layer 13. This has the result of changing signal phase and shifting the resonance. The biosensor response is shown in Figure 2. Here the relative phase of the detected signal is depicted as a function of the elevation angle ψ of source S and detector D. Curves (a) to (c) show the different responses obtained when the changes Δt_3 of layer thickness t_3 are zero, +5nm, and +10nm respectively. In each case the phase changes

by 2π as source and detector are swept across the resonance. At a fixed elevation angle ψ_i the change Δt_3 in layer thickness t_3 is discernible as a change in phase $\Delta\phi$.

It is a problem that changes in phase can result from other causes. In such circumstances reliance upon absolute measurement of changes in phase can lead to false detections and to errors in any quantitative analysis based upon such measurements. In particular, it is noted that changes in ambient temperature result in dimensoinal changes in the resonant monitored cavity and also result in a resonant shift. An example of the effect of such changes is illustrated by curve (d) of Figure 2. Here it has been assumed that the change Δt_2 of the thickness t_2 of the cavity body 12 is +10nm. It can be seen that the shift of the resonance is appreciable. Surface irregularities and mirror misalignment also have a contributory effect.

DISCLOSURE OF THE INVENTION

The present invention is intended as a solution to the problem aforesaid. It is an objective to provide phase related measurements that are appreciably insensitive to changes of ambient temperature and the like.

In accordance with a first aspect of the present invention there is provided a method for detecting optical phase changes during biosensor operation, this method comprising the following steps:

providing a resonant optical biosensor in which a coating layer thereof is in part sensitized to at least one given assay species and is patterned for causing light diffraction;

exposing the patterned sensitized coating layer to a fluid sample;

directing light from an extended light source towards, and resonantly coupling the same to, the patterned sensitized coating layer;

collecting and focussing light diffracted from the patterned sensitized coating layer onto a light detector, defining thus a diffraction field in the plane of the light detector; and,

scanning the light detector relative to the diffraction field to measure the same and thereby detect or quantify said at least one given assay species when such is present in the fluid sample.

In the absence of a given assay species being present in the fluid sample, that part of the coating layer that is sensitized is not optically distinguished from the remainder part of the coating layer. In these circumstances, no diffraction field is discernible. Once a given assay species is present in the fluid sample, however, this species is attracted to the layer and results in an increase in thickness in that part of the coating layer that is sensitized. Localised shift in phase, light interference and diffraction are the result. The presence of a measurable diffraction field is thus an indicator of the detection of a given assay species. The quantity of the given assay species present in the fluid sample may be determined from an analysis of the diffraction field measurements.

It may be determined, for example, from measurement of first order diffraction spot position and size, or position and intensity. The latter is preferred as being less sensitive to surface imperfection.

The method aforesaid may be extended to allow the detection and quantification of one or both of two different assay species present in the fluid sample. Useful information can be extracted

from measurement and comparison of the position and intensity of first and higher order diffraction spots, as will be discussed hereinafter.

It will be noted that the diffraction field is not significantly altered by changes in biosensor geometry. It is primarily determined by the configuration and thickness profile of the coating layer.

In accordance with a second aspect of the present invention there is provided biosensing apparatus suitable for performing the method aforesaid, this apparatus comprising:

a resonant optical biosensor in which a coating layer thereof is in part sensitized to at least one given assay species and is patterned for causing light diffraction;

an extended light source;

collimating means arranged to direct light from the extended light source onto the resonant coupled optical biosensor;

a light detector;

light focussing means arranged to direct light diffracted from the patterned sensitized coating onto the light detector thereby to define a diffraction field in the plane of the detector; and,

scanning means arranged to scan the light detector relative to this diffraction field.

In the apparatus aforesaid the diffraction field may be scanned mechanically. To this end the light detector may consist of a linear array of detecting elements aligned in elevation, and the light source and light detector may be moved each in a transverse direction to effect a scan in azimuth. In this arrangement it is convenient to

adopt a light source consisting in a linear array of light emitting diodes also aligned in elevation.

Alternatively, the field may be scanned electronically. The light detector and the light source may each consist in a two-dimensional array of elements, which elements are switched column to effect a scan in azimuth.

In accordance with a third aspect of the present invention there is provided a resonant optical biosensor including a coating layer, wherein this coating layer is in part sensitized to at least one assay species and is patterned for causing light diffraction.

The coating layer aforesaid may be in part sensitized to a single given assay species. In this instance the coating layer may be sensitized and patterned with a set of longitudinally extending stripes, alternate stripes thereof being sensitized to the single given assay species.

Alternatively, and for more complex detection and analysis, the coating layer aforesaid may be in part sensitized to two or more different assay species. The coating layer thus may have a first part sensitized to a first assay species, a second part sensitized to a second assay species, and a remainder third part. In this instance the coating layer may be sensitized with adjacent sets of first, second and third longitudinally extending stripes in which the first and second stripes are sensitized to the first and second assay species respectively and in which the first and second stripes are of different width.

BRIEF INTRODUCTION OF THE DRAWINGS

In the drawings accompanying the specification:

Figure 1 is a cross-section drawing of biosensing apparatus including a biosensor of known construction;

Figure 2 is a graph in which the phase of a detected signal in the apparatus of Figure 1 preceding is shown as a function of the elevation angle subtended by both source and detector for different values of layer thickness;

Figure 3 is a perspective view of biosensing apparatus in which the sensitized coating layer of the biosensor has been modified in accord with the present invention;

Figure 4 is a plan view of a modified sensitized coating layer, a variant of that shown in the preceding figure; and;

Figures 5 and 6 are diffraction spot patterns obtained using the apparatus of Figure 3 in which the coating layer is as provided and as modified as shown in Figure 4, respectively.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

So that this invention shall be better understood, embodiments will be described now and reference will be made to Figures 3 to 6 of the drawings. The description that follows is given by way of example only.

In the biosensing apparatus shown in Figure 3, the resonant optical biosensor 1 has been modified by substituting an alternative coating layer 13' for the uniformly sensitized coating layer 13 described above. The area of the substituted layer 13' is sensitized in part only. It serves as a means of imposing diffraction in the detected light field. A convenient form of sensitized pattern is illustrated in which the area of the substituted layer 13' is divided into a series of longitudinally extending stripes, stripes 15

corresponding to a part of the layer material that is sensitized to the given assay species, and stripes 17 (alternate with the stripes 15) corresponding to the remainder part of the layer material which is not so sensitized and serves to act as a localised phase reference. The patterned layer 13' may be formed from a sensitized material in which that part corresponding to the reference stripes 17 is desensitized selectively. The biosensor is illuminated by a divergent beam produced using a collimating lens 19 and an extended light source S'. The light source S' extends a significant distance in elevation to produce an appreciable beam divergence $\Delta\psi$. Light emerging from the biosensor is collected by a telescopic sight 20 and is focussed onto a light detector D' located at the focal plane.

In the absence of the given assay species being present in the fluid sample, no change occurs in the thickness of either part of the patterned layer 13'. Each part contributes equally to the change of phase in the detected signal. No appreciable interference and no significant diffraction field is produced at the focal plane of the telescopic sight 20.

In the presence of the given assay species in the fluid sample, the stripes 15 of sensitized material increase in thickness and become optically distinguishable from the reference stripes 17. Optical interference results and a diffraction pattern becomes discernible at the focal plane of the telescopic sight 20. This is shown in Figure 5 where for clarity the zero order and first order diffraction spots only are shown. The detailed structure of the diffraction pattern is dictated by the stripe geometry and is dependant upon the relative thickness of the sensitized and reference stripes 15 and 17.

The diffraction pattern is measured by scanning the detector D' in azimuth ø across the diffraction field. Intensity and azimuth data is collected and used for quantitative analysis. More complex detection and analysis may be performed by simple modification of the biosensor described. It is possible to afford detection and quantitative analysis for two distinctive assay species simultaneously. To this end the coating layer is provided with a first part that is sensitized to a first assay species, a second part that is sensitized to a second array species, and a third remainder part. One possible configuration is shown in Figure 4. The area of the coating layer 13" is, in this instance, partitioned by repeating sets of three longitudinally extending stripes 21, 22, 23. The first of these stripes, stripe 21, is sensitized to the first assay species. The second of these stripes, stripe 22, is sensitized to the second assay species. The remainder stripe 23 has no such sensitization and provides a reference. In order to avoid ambiguity in the results, the sensitized stripes 21 and 22 are of different widths. In the illustration, stripes 22 are the wider.

Consider now how such a biosensing apparatus would respond when the patterned coating is exposed to a fluid sample. When neither the first nor the second assay species is present, the parts of the coating layer 13" will be indistinguishable and no diffraction pattern will be discernible. However, when either the first or the second assay species is present, the corresponding stripes 21 or 22 respectively will become active, i.e. increase in thickness. The result, as before, is a diffraction pattern. A section of the diffraction pattern is illustrated in Figure 6 in which the zero order, first order and next

higher order secondary diffraction spots are shown. The diffraction patterns for the first and second assay species will be quantitatively similar. The diffraction patterns however can be distinguished. It will be recalled that stripes 21 and 22 are of different widths, stripes 22 being wider. For the second assay species the first order diffraction spots will be dimmer relative to the zero order diffraction spot. The form of this decline is:

$$\frac{1}{n} \text{ Sin } \frac{2\pi \text{ wn}}{W}$$

where n is the order of the outlier (1, 2 etc); w is the width of the corresponding stripe, and W is the spatial repeat period. It is noted that spots of the same order to the left and to the right of the diffraction pattern are of equal intensity.

Should both first and second assay species be present, both stripes 21 and 22 will be active. However the diffraction pattern resulting is distinguishable from the foregoing in that spots of the same order to the left and to the right of the diffraction pattern will no longer be of equal intensity. An exception to this rule arises in the event that both stripes 21 and 22 result in equal phase shift. Even in this case the diffraction pattern is distinguishable from the diffractions patterns for either the first or second assay species, because of the different geometry of the effective layer pattern. Thus where w_1 and w_2 are the widths of stripes 21 and 22, the appropriate value for the width w used in the above formula is $w_1 + w_2$ as compared with w_1 or w_2 when first or second assay species above are present. In summary it can be seen therefore that it is

possible to distinguish events in which none, one or both first and second assay species are present.

Data collected for intensity and angular position can be used in numeric analysis (deconvolution) to provide quantitative information.

CLAIMS:

1. A method for detecting optical phase changes during biosensor operation, this method comprising the following steps:

providing a resonant optical biosensor in which a coating layer thereof is in part sensitized to at least one given assay species and is patterned for causing light diffraction;

exposing the pattern sensitized coating layer to a fluid sample;

directing light from an extended light source towards, and resonantly coupling the same to, the patterned sensitized coating layer;

collecting and focussing light diffracted from the patterned sensitized coating layer onto a light detector, defining thus a diffraction field in the plane of the light detector; and,

scanning the light detector relative to the diffraction field to measure the same and thereby detect or quantify said at least one given assay species when such is present in the fluid sample.

2. A method as claimed in Claim 1, in which the coating layer includes a first part that is sensitized to a first assay species and a second part that is sensitized to a second assay species.

3. A method as claimed in Claim 2, in which the coating layer is partitioned by repeating sets of three longitudinally extending stripes, the first stripes being sensitized to a first assay species, the second stripes being sensitized to a second assay species, and the third stripes having no sensitization and providing a reference.

4. A method as claimed in Claim 3, in which the first and second sensitized stripes are of different width.

5. A method as claimed in Claim 1, in which the coating layer is sensitized and patterned with a set of longitudinally extending stripes, the alternate stripes being sensitized to a single assay species.

6. A biosensing apparatus comprising:

a resonant optical biosensor in which a coating layer thereof is in part sensitized to at least one given assay species and is patterned for causing light diffraction;

an extended light source;

collimating means arranged to direct light from the extended light source onto the resonant coupled optical biosensor;

a light detector;

light focussing means arranged to direct light diffracted from the patterned sensitized coating onto the light detector thereby to define a diffraction field in the plane of the detector; and,

scanning means arranged to scan the light detector relative to this diffraction field.

7. An apparatus as claimed in Claim 6, wherein the light detector includes a linear array of detecting elements aligned in elevation, the light source and the light detector being movable in a transverse direction to effect a scan in azimuth.

8. An apparatus as claimed in Claim 6, in which the light source is a linear array of light emitting diodes.

9. An apparatus as claimed in Claim 6, in which the light detector and the light source, each includes a two dimensional array of elements, which elements are switched column to effect a scan in azimuth.

10. An apparatus as claimed in any one of claims 6 to 9, in which the coating layer includes a first part that is sensitized to a first assay species and a second part that is sensitized to a second assay species.

11. An apparatus as claimed in Claim 10, in which the coating layer is partitioned by repeating sets of three longitudinally extending stripes, the first stripes being sensitized to a first assay species, the second stripes being sensitized to a second assay species, and the third stripes having no sensitization and providing a reference.

12. An apparatus as claimed in Claim 11, in which the first and second sensitized stripes are of different width.

13. An apparatus as claimed in anyone of Claims 6 to 9, in which the coating layer is sensitized and patterned with a set of longitudinally extending stripes, the alternate stripes being sensitized to a single assay species.

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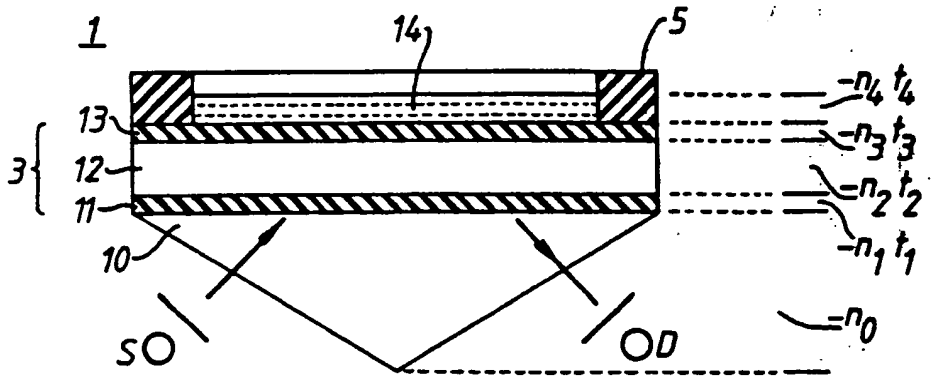
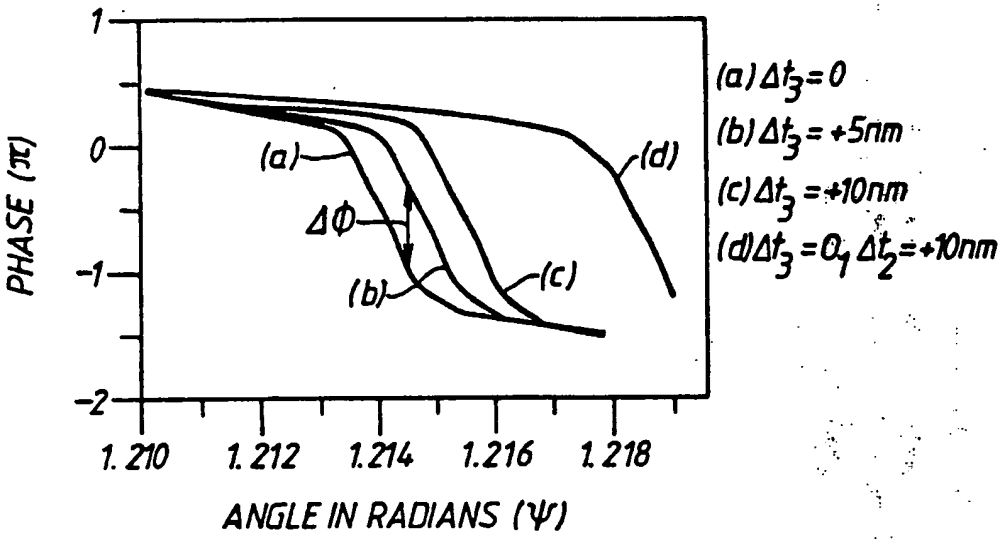


PRIOR ART

Fig.1.



PRIOR ART

Fig. 2.

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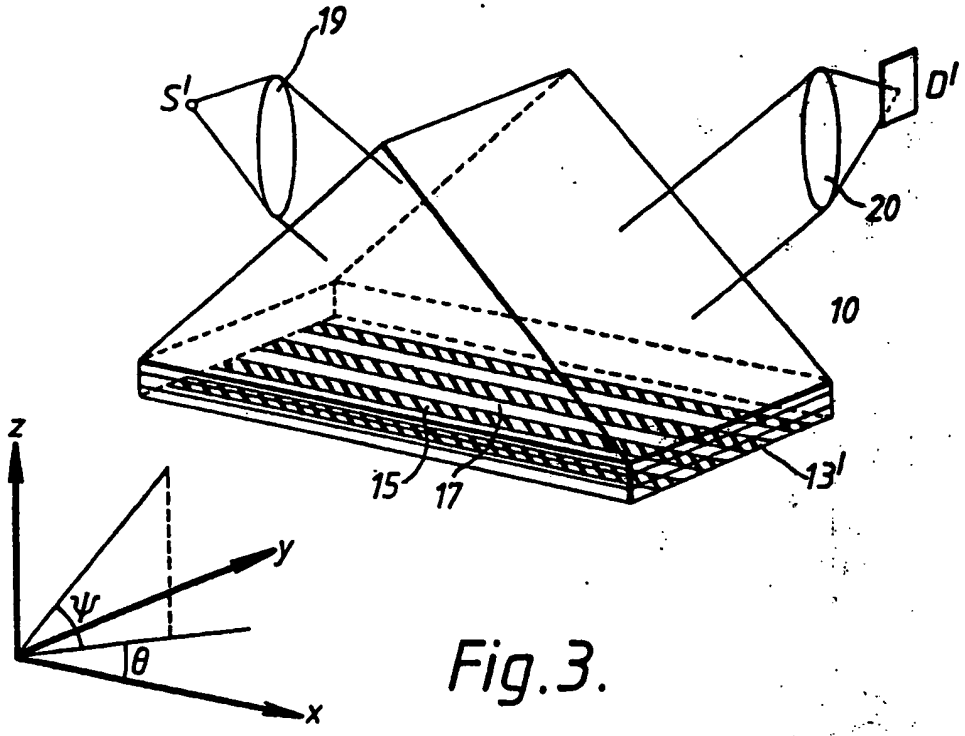

Fig.3.

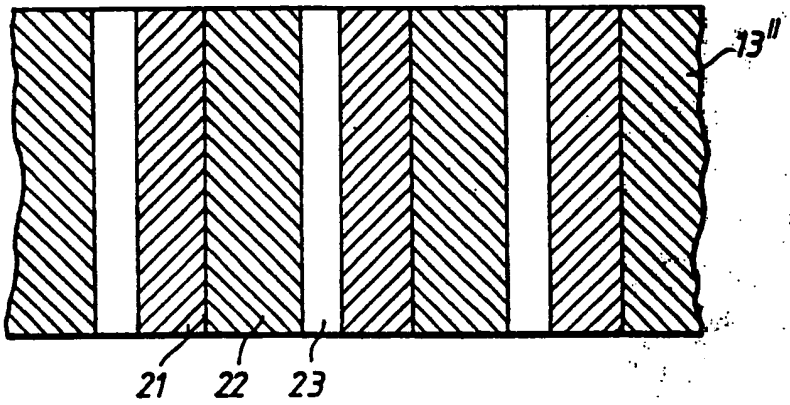

Fig.4.

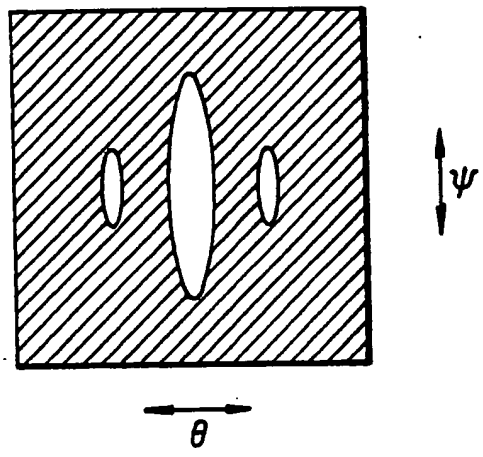

Fig.5.

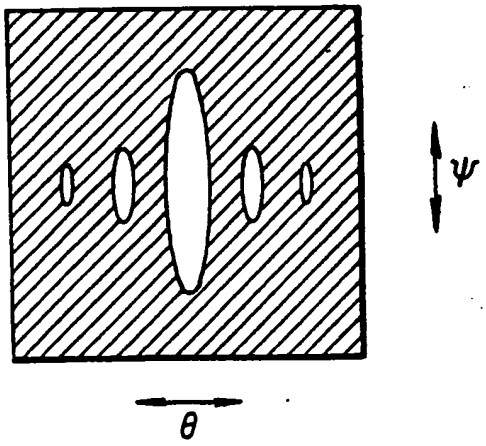

Fig.6.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/00119

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 21/75

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
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Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	WO, A1, 84/02578 (COMTECH RESEARCH UNIT LIMITED) 5 July 1984, see page 10, line 7 - line 13; abstract; figure 1	1,6
A		2-5,7-13
	--	
X	US, A, 4647544 (D.F. NICOLI ET AL) 3 March 1987, see abstract; figures 1-3	1,5,6, 13
A		2-4,7-12
	--	
X	US, A, 4537861 (V.B. ELINGS ET AL) 27 August 1985, see column 12, line 40 - line 52; column 13, line 4 - line 12; abstract; figure 4	1,5,6, 13
A		2-4,7-12

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECONT SHEET)

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A		2-4,7-12
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X	EP, A3, 0194132 (MUREX CORPORATION) 10 September 1986, see page 10, line 1 - line 6; abstract; figure 1	1,6
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 90/00119

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		EP-A-	0117988	12/09/84
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